Chlamydomonas reinhardtii data bases. The isomerization of the *all-trans*-retinal chromophore induces conformational changes of the protein that result in the opening of the channel pore to allow ion flow across the membrane. ChRs attract enormous attention because after expression in neuronal cells they can trigger action potentials upon blue light stimulation (450 nm). In 2007, we isolated two new ChRs from the fresh water algae *Volvox carterii* (VChR1 and VChR2). Notably VChR1 shows a red-shifted action spectrum peaking at 535 nm. Thus, VChR1 can be used to trigger action potentials in neurons by yellow light illumination [3]. Expression of ChR2 and VChR1 in different neuronal species enabled a distinct activation of the two cell types with blue and green light helping to understand neuronal circuits. But, a broader application of VChR1 was hampered because of its poor membrane localization and small currents in neurons. In this study we developed a well-expressing ChR with absorption characteristics similar to VChR1 by combining the N-terminal part of ChR1 and the C-terminal part of VChR1.

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APBSmem: A Tool for the Analysis of Membrane Protein Electrostatics

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Electrostatic forces orchestrate the folding of proteins, increase the binding of one protein to another and facilitate protein-DNA and protein-ligand binding. A popular means for computing the electrostatic properties of biological systems is to numerically solve the Poisson-Boltzmann (PB) equation, and there are several easy-to-use software packages available for carrying out these calculations on soluble proteins. We have developed a tool called APBSmem that performs these calculations in the presence of a membrane. Adaptive Poisson-Boltzmann Solver (APBS) is used as a back-end for solving the PB equation, and a graphical user interface (GUI) coordinates a set of routines that introduce the influence of the membrane, determine its placement and shape relative to the protein, and set the membrane potential. The software Jmol is embedded in the GUI to visualize the protein inserted in the membrane and the resulting electrostatic potential. We demonstrate the use of our software with three examples involving the calculation of the protein transfer free energy from water to membrane, solvation energy required to move an ion into a channel, and the gating charge of a molecular motion. We expect that the ease with which the GUI allows one to carry out these calculations will make this software a useful resource for experimenters and computational researchers alike. In particular, our built-in protocols should be appealing to researchers studying ion channel and transporter function.

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The Pivotal Twin-Histidine Element of the Escherichia Coli Ammonium Channel AmtB Functions as a Substrate Selectivity Filter

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Ammonium functions as both a primary nutrient and waste product and, thus, its transport across biological membranes is of fundamental importance. Because the uncharged form, NH_3, readily traverses phospholipid bilayers by simple diffusion the role of protein-catalyzed transport of the protonated species, NH_4^+, is unusually interesting. The Amt family of channels mediates the transport of NH_4^+ and is required for microbial growth when diffusion of NH_3 becomes limiting for nitrogen uptake. Whereas all other characterized channels facilitate downhill substrate movement, Amt proteins are active channels - hybrids between passive channels and active transporters - and concentrate NH_4^+ against a gradient. Amt family members function as homotrimers, with each monomeric unit carrying a pore for substrate conduction. Each pore is lined entirely with hydrophobic residues, save for a pair of conserved hydrogen-bonded histidines postulated to play a critical role in mediating NH_4^+ transport. We examined the impact that changes to this histidine pairing had on the function of one of the best-characterized members of the Amt family, the AmtB protein of *Escherichia coli*. Our initial analysis indicated that AmtB can accommodate, by either direct substitution or suppressor generation, acidic residues at one or both positions of the twin-histidine site while retaining good-to-excellent transport activity. Subsequent work shows that a number of mutant AmtB proteins carrying such alterations leak K^+ ions and that this leakage is energetically costly. These findings lead us to conclude that whereas the twin-histidine element is not required to conduct NH_4^+ it serves as a filter to prevent AmtB-mediated K^+ transport.

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Mapping Water Density to Design New Blockers Against a Viral Proton Channel

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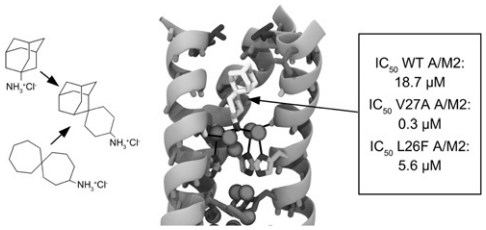
The influenza A virus utilizes a membrane-embedded proton channel, M2. Its functions are to acidify the viral interior and trigger the uncoating of its RNA, equilibrate the pH across the Golgi during replication, and allow a fully formed virus to bud from the host cell. One of the two classes of approved anti-influenza drugs contains amphiphilic molecules such as amantadine, that prevent extraviral H+ and water from accessing the pore of M2. Drug-resistant mutations (now pervasive through most of the flu strains) feature a more hydrated pore that destabilizes amantadine binding. We have used molecular dynamics simulations over extended times, reconstructed accurate water density maps, and identified several metastable positions of amantadine in the wild-type protein and its mutants. Using these data we designed new amine-based inhibitors, that fully suppress H+ conduction and viral replication in drug resistant strains.



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Effects of Substance P on Excitability of Dorsal Root Ganglion Neurons

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Some primary nociceptor neurons produce and release substance P (SP), a peptide neurotransmitter with well-described effects on second-order sensory neurons. However, the effects of SP on primary sensory neurons are less clear. We tested the hypothesis that SP acts on an autoreceptor by examining the pharmacological profile of rat DRG neurons sensitive to SP. Whole-cell patch clamp was used to measure the response of 89 cells to brief applications of pH 7 (27 positive cells), pH 6 (37 positive cells), capsaicin (22 positive cells), and ATP (15 P2X3 type positive cells). Sensitivity to SP was determined by an increase in cell excitability measured as the number of action potentials at the threshold and the slope of the stimulus-response curve (16 positive cells). There was also a decrease in excitability in 8 cells. Among the cells responding to SP by increasing excitability, the frequencies of sensitivity to pH 7 (67%) and pH 6 (88%) were higher than in non-responding cells (24% and 32 % respectively, $p<0.01$). The frequencies of sensitivity to capsaicin had a tendency to be higher in the SP responding cells (38% versus 22%, $p=0.33$). P2X3 type ATP currents were present in 15 of 73 (21%) SP non-responding cells, while none of the 16 SP positive cells presented this current ($p=0.1$). We conclude that the majority of SP sensitive neurons exhibit a pharmacological profile typical of nociceptors, although P2X3 currents were not present in these cells.

3462-Pos Board B323

Ion Channel Proteins that Spontaneously Insert into Lipid Bilayer Membranes: An Impedance Spectroscopy Study Employing Tethered Membranes

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The Chloride Intracellular Ion Channel (CLIC) protein family, the Annexins, the bacterial toxins Hemolysin (AH), Streptolysin, Perfringolysin Listeriolysin, Pneumonlysin, Ivanolysin and Colicins all possess unusual mechanisms for inserting into cellular and/or host membranes. They are representatives of proteins that spontaneously insert into membranes, by-passing the route for the incorporation of most integral membrane proteins. For example, the soluble form of the 240-amino acid polypeptide CLIC1 is known to exist in at least two conformations due to a large rearrangement of its amino terminus under the influence of oxidation. Oxidation promotes its binding to and insertion